

## Article

# Development and Metabolic Characterization of Horse Gram (*Macrotyloma uniflorum* Lam. (Verdc.)) Mutants for Powdery Mildew Resistance

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**Abstract:** Horse gram is one of the lesser-known beans widely grown in India. One hundred and twenty-three homozygous horse gram mutants were screened for powdery mildew (PM) disease resistance using the grade 0 to 4. The mutants were grouped based on the disease level of 0 to 2 (resistant) and susceptible (3 to 4). The PM altered the chlorophyll fluorescence (a/b ratio), maturity duration, and yield attributing traits. The yield loss ranged from 4.55% to 72.66%. After affirming the resistance level, the resistant mutant (RM) with minimum yield loss (scale:0) and the susceptible mutant (SM) with maximum loss (scale:4) were used for metabolomic analysis through GC-MS. PM infection induced expression of 66 metabolites representing 32 functional classes. The number of unique classes in RM and SM was 13 and 11, respectively, while eight were common. A fold change in the common metabolites indicated an enhanced accumulation of amine, alcohol, and ester in RM. Along with pathogen-induced defensive metabolites, RM produced silane and fluorene, whose biological significance in disease resistance is unknown. Though SM expressed defence-related bio-molecules, it failed to yield better.

**Keywords:** *E. polygoni*; GC-MS; horse gram; metabolomics

## 1. Introduction

Legumes are cultivated in two main seasons, *Kharif* (June–September) and *Rabi* (September/October–December) in southern India. Legumes, such as green gram, black gram, and red gram, are usually cultivated during the *Kharif* season. The *Rabi* season coincides with the winter season, and therefore dew-loving legumes, such as chickpea and horse gram, are commonly grown. Generally, the rainy days are few during the *Rabi* season, reinforcing the cultivation of medium-duration and less water-dependent crops such as arid legumes. Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) is one such important diploid, cool-season arid legume cultivated in dry and marginal lands. This legume is grown as a contingent crop in accordance with the completion of the north-east monsoon (*Rabi* season) to utilize the residual moisture. This crop is preferred by the marginal farmers with poor land resources owing to its multi-utility [1]. Horse gram

cultivation accomplishes many functions, such as (i) sustains nutritional sustainability in the arid region; (ii) breaks the cereal disease continuum; (iii) enriches the soil nitrogen through root nodules; (iv) enriches the soil organic carbon through leaf litter; (v) avoids soil erosion as a cover crop; and (vi) produce nutritious fodder. Horse gram can reclaim cultivable wastelands into a Nutri garden. The nutrient pre-eminence of horse gram includes significant protein, lysine, and micronutrient contents. Being a neglected crop, it provides many nutrients, as it is highly essential for humans. It prevents us from various types of dreadful diseases. The therapeutic potential of horse gram has been well retrieved from the ancient literature [2]. Strict self-pollination, narrow genetic variability for economic traits, and fewer studies on germplasm characterization urge accelerated breeding programs in horse gram as it has been identified to be a promising crop of the future [3]. The success of classical hybridization to create genetic variation in horse gram is limited due to its small flowers, closed floral structure, prostrating growth habit, and significant flower dropping. The potential of induced mutagenesis in the evolution of the variability in economic traits for horse gram has been reported [4]. Powdery mildew challenges photosynthesis and alters routine physiological processes, thereby significantly reducing horse gram yield [5].

Plants express stress-responsive transcripts and synthesize compatible metabolites to sustain stressed conditions [6]. Extensive transcriptomic studies were carried out to understand the role of different metabolic pathways under stress [7]. Metabolomics is an authoritative tool that assists in analysing the stress-induced metabolic changes that supplement the transcriptome data and thus helps develop or identify stress-tolerant genotypes. Metabolite profiling through the GC-MS technique is widely used to understand the metabolite changes under stress [8]. Stress-induced metabolic changes act as osmolytes, antioxidants, chelating agents, maintain cell membranes, iron transport, and turgidity, and also stabilize proteins and enzyme biosynthesis [9]. These up/down regulations of biomolecules are the energy budgeting strategies evolved by plants to deal with the stress [10]. Reports on the resistance sources for *E. polygoni* in horse gram are rare.

Further, the knowledge on *E. polygoni*-induced metabolic changes is still unexplored. The tolerance mechanism for *E. polygoni* can be deciphered through comparative metabolomic studies between resistant and susceptible genotypes. Therefore, the present study aimed to classify the horse gram mutants into different powdery mildew resistant groups and comparative metabolomic profiling of resistance for further utilization.

## 2. Materials and Methods

### 2.1. Genetic Material and Categorization for Powdery Mildew Resistance

#### 2.1.1. Evolution of Genetic Materials

Two mutant populations were developed by mutating the popular horse gram varieties PAIYUR 2 and CRIDA 1–18 R.

The mutagens were gamma rays (G) (100–400 Gy), electron beam (EB) (100–400 Gy), G + EB (100–400 Gy), and a combination of G and ethyl methanesulfonate (EMS) (G:100–400 Gy + EMS: 0.3%). In each treatment, 500 seeds were mutated. The M<sub>1</sub> and M<sub>2</sub> generations were sown during *Rabi* 2017 and 2018, respectively. The mutant populations were handled separately. All the M<sub>1</sub> plants were harvested individually and forwarded to M<sub>2</sub>. The mutagenic efficiency and effectiveness were worked out based on the chlorophyll mutants and injuries/sterility. The promising M<sub>2</sub> segregants were harvested individually and forwarded on a plant to row basis to M<sub>3</sub> during *Rabi* 2019. The M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub> experiments were conducted at the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India.

#### 2.1.2. Screening of Mutants for Powdery Mildew Resistance

Of the total mutants evolved, a total of 123 homozygous M<sub>3</sub> promising mutants were alone considered for powdery mildew resistance screening under natural epiphytic conditions (Table S1) during the *Rabi* (winter) season 2019. The climatic parameters are shown in Table S2.

The statistical design and replications were randomized block design (RBD) and three, respectively. All the recommended packages in current practices were followed. The conidia of *E. polygoni* were collected from the infected plants raised under greenhouse condition in sterile water using a soft brush. A suspension of conidia containing about  $1 \times 10^6$  per mL was prepared separately in sterile water using a haemocytometer. The 25-day-old crop was inoculated by spraying this suspension with a hand sprayer at 4 PM for powdery mildew infection at vegetative growth. An equal number of plants were left un-inoculated to serve as a control. The plots were not sprayed with plant protection chemicals. The mutants were classified into varied resistant classes based on a 0–4 scale, as advocated by [11–13]. All plants in replication were considered for grading 17 days after inoculation. The description of the grading is given in Table 1.

**Table 1.** Details of the scaling technique adopted for powdery mildew resistance screening in horse gram.

Description	Score	Disease Reaction
No symptoms of powdery mildew growth	0	R
Small and sparse mycelial growth. Visualization of non-significant sporulation	1	R
Macroscopic: Slight mycelia growth Microscopic: Slight to moderate mycelia growth with conidiophores	2	R
Macroscopic: Moderate mycelial growth Microscopic: Moderate mycelial development with moderate to heavy sporulation	3	S
Macroscopic: Heavy mycelia growth Microscopic: Heavy mycelia growth with copious sporulation	4	S

### 2.1.3. Pathogen Confirmation and Classification of Mutants

The conidial confirmation and density observation were done using a phase-contrast microscope (PCM) and scanning electron microscopes (SEM). For the SEM analysis, the samples were coated with a low-vacuum sputter coating machine (EMITECH (SC7620) and mounted on a specimen stub. The FEI QUANTA 250 instrument was used for fruiting body identification. Five plants from each mutant were randomly tagged, and per plant yield was calculated. The percent yield loss was calculated by the formula: yield of inoculated mutant/yield of respective control  $\times 100$ . This preliminary data was utilized to select extreme mutants for further testing under both fields and controlled conditions in the subsequent generation. The resistant (scale 0, 1 and 2) and susceptible (scale 3 and 4) group mutants were harvested separately.

### 2.1.4. Confirmation of Powdery Mildew Resistance and Tagging of Mutants for Metabolomic Analysis

In the subsequent year, the powdery mildew resistance of the tagged mutants was re-established by both confirmative field and shade-net trails during *Rabi* 2020. The experiments were conducted at the Sugarcane Research Station, Melalathur, Tamil Nadu Agricultural University. The weather parameters are shown in Table S2. For the confirmative field trial, a field grown with horse gram in the previous season and in situ ploughed with powdery mildew infected residues was selected. A total of three isolated resistant (scale:0) and nine susceptible (scale:4) mutants were sown in three replications in RBD. Each entry was sown in five rows. The susceptible mutant with maximum yield loss in the previous year's trial (TNAU-HG-M004) was sown in two rows after every resistant entry to ensure adequate inoculum load. To affirm disease prevalence at the appropriate crop growth stage, TNAU-HG-M004 was sown twenty days earlier than the resistant mutants. The same set of entries without inter-planting of TNAU-HG-004 was sown in a control plot. The conidium collected from TNAU-HG-M004 was tapped over other mutants at the

vegetative stage. During the study period, no plant protection chemicals were sprayed. To estimate the powdery mildew induced economic loss, the yield and its attributing traits were recorded in five randomly selected plants in all replications. The same set of mutants was sown in pots as a second confirmative trial in a completely randomized block design in a shade-net study. The soil from the inoculum-loaded experimental field was utilized to prepare the potting mixture. Each entry was replicated thrice, and five pots were sown per replication. At the vegetative stage, powdery mildew-infected leaves of TNAU-HG-004 were stapled on ten randomly selected leaves of other mutants. The scaling technique for both the field and pot studies was adopted as described in Table 1. Five plants from each mutant were randomly tagged, and the powdery mildew induced changes in chlorophyll fluorescence (a/b ratio), crop maturity duration, and yield were documented. The chlorophyll fluorescence (a/b ratio) was estimated using an OS1p+ chlorophyll fluorometer (Optic sciences). At harvest, the resistant mutant with the minimum yield loss and the susceptible mutant with the maximum yield loss were tagged, compared, and utilized for metabolomic studies through GC-MS.

#### 2.1.5. Data Analysis

The data pertinent to grouping and tagging of extreme mutants were analysed using SPSS version 12 for estimating the standard error of the mean.

#### 2.2. Metabolite Extraction and GC-MS Analysis

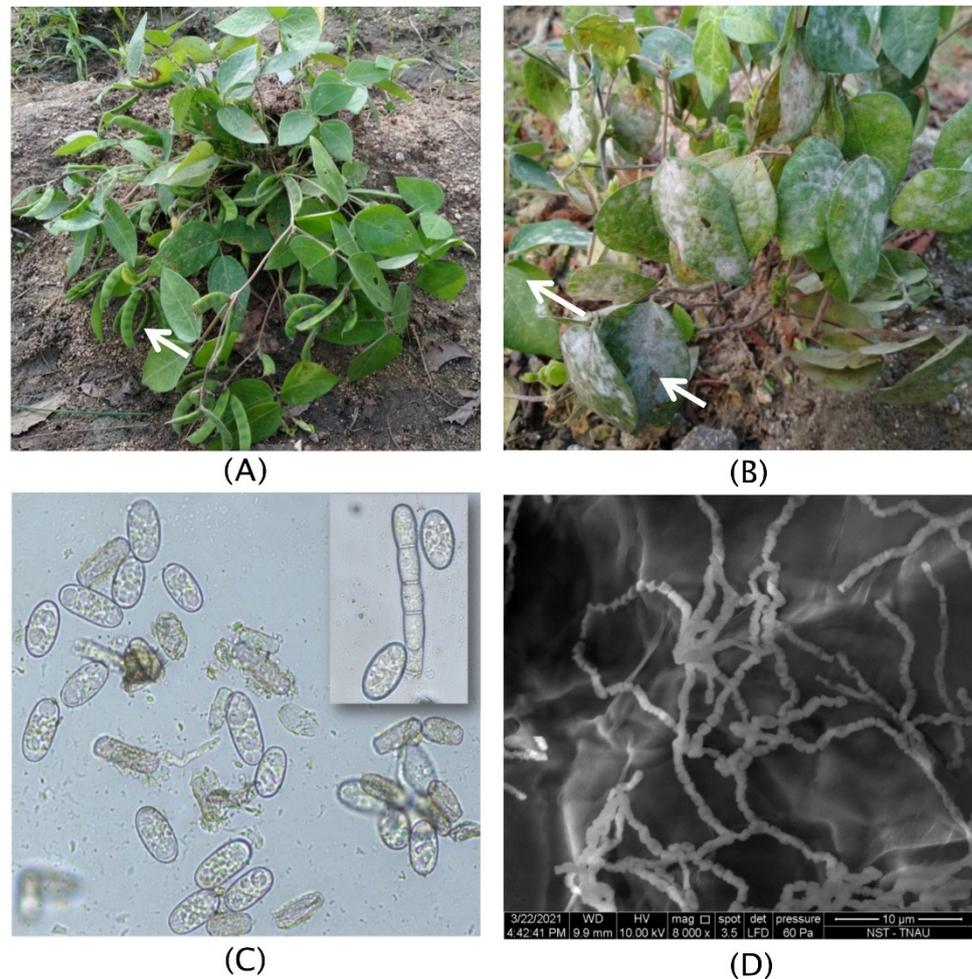
Leaf samples from the pot-grown resistant mutant with minimum powdery mildew-induced yield loss (TNAU-HG-M11) and the susceptible mutant with maximum yield loss (TNAU-HG-M4) were collected after 17 days of inoculation for metabolomic analyses. Leaf tissues in three replications immediately after collection were flash-frozen in liquid nitrogen. Fresh frozen leaf tissues (40 mg) were ground using liquid nitrogen, extracted with methanol followed by chloroform [14]. An aliquot of 50  $\mu$ L ribitol (0.2 mg/mL in water) was utilized as internal standard and filtered through a PVDF syringe filter (0.22  $\mu$ m) (Millipore, Dublin, Ireland). Speed Vac was used to concentrate the extracts. N-methyl-N-[trimethylsilyl] trifluoroacetamide (80  $\mu$ L; MSTFA) was used for trimethylsilylation. The temperature and time of trimethylsilylation were 37  $^{\circ}$ C and 30 min, respectively. The samples (about 1  $\mu$ L) were injected into a GC injection port (AI3000 II, Thermo Fischer Scientific, Waltham, MA, USA) connected to a GC-MS (TRACE<sup>TM</sup> GC Ultra with DSQII Quadrupole mass spectrometer). The technical specifications of the capillary column are 30 cm length, 0.25 mm diameter, and 0.25  $\mu$ m film thickness-Agilent, DB-5MS Ultra Inert, and 122–5532. The injection port temperature was maintained at 250  $^{\circ}$ C. The oven temperature was programmed at 70  $^{\circ}$ C (5 min hold) and increased 5  $^{\circ}$ C per minute to 300  $^{\circ}$ C. Helium was the carrier gas, and the flow rate was 1 mL/s. The temperature at the electron ionization source was maintained at 250  $^{\circ}$ C. The abundance of ions in the range of 50–600  $m/z$  was scanned at a rate of 1.1 scans per second. The NIST library was used to identify the compounds, and the data were analysed using R software.

### 3. Results and Discussion

#### 3.1. Powdery Mildew-Induced Changes in the Yield Attributing Traits

A total of 123 homozygous horse gram mutants (Table S1) were screened for powdery mildew resistance under field conditions during the *Rabi* season 2019. The mutants were free from powdery mildew infection up to 35 days after sowing. The infection started when a shading effect formed between leaves in the cool dew days. The climatic conditions are provided in Table S2. Initially, small, white, powdery spots appeared on the upper surface of the leaves, and the size of the spots progressed along with the age of the crop. Later, the infection spreads onto the lower leaf surface; the leaves become brown, brittle, the petiole becomes weak, broken at the joints, and the leaves are forced into premature senescence (Figure S1a–f). During the third stage of infection, a white powdery mass developed on the pods (Figure S2a,b). A visible difference was noticed between the

resistant and susceptible genotypes (Figure 1A,B). From the susceptible mutant, white powdery masses were collected for species identification. Based on the morphology of the conidiophores and conidia of the pathogen, it was identified as *E. polygoni* (Figure 1C,D). It was observed that barrel-shaped conidia were borne on the conidiophores in chains. The conidiophores were short, initially cylindrical, and later changed to barrel-shaped, and became elliptical at disposal.

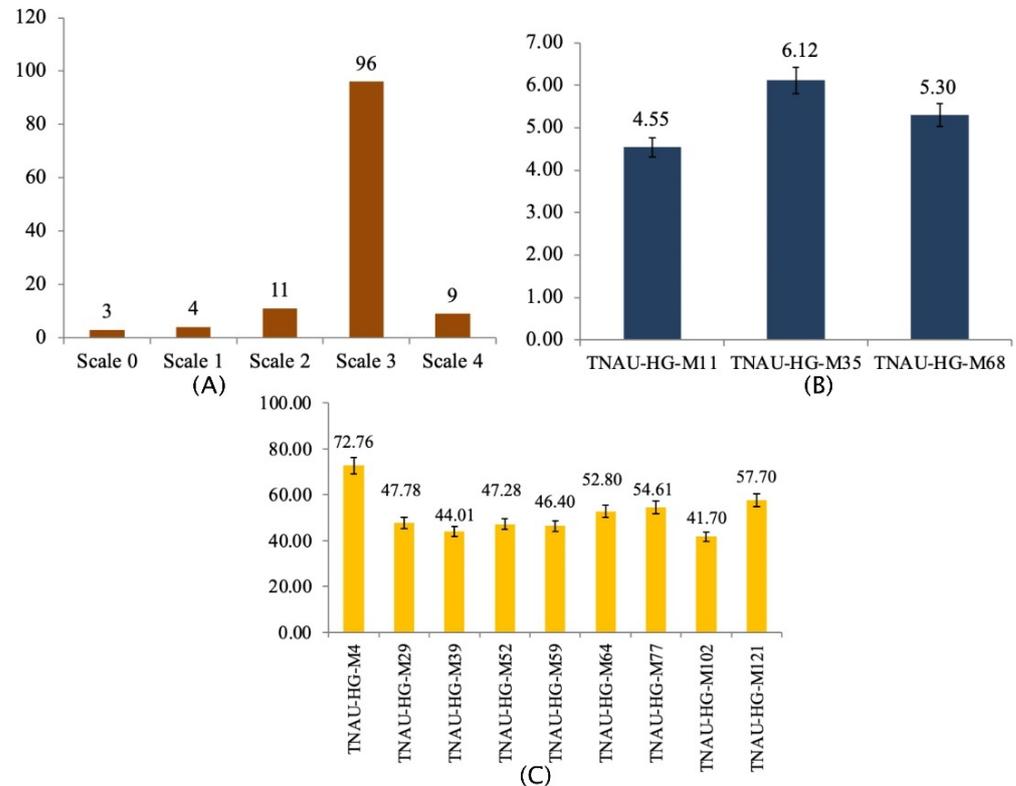


**Figure 1.** (A) Powdery mildew-resistant mutant; (B) powdery mildew-susceptible mutant; (C) Identification of the powdery mildew species: phase-contrast microscopy; (D) Identification of powdery mildew species: SEM.

Based on the level of infection, the mutants were grouped into four categories: Scale 0, Scale 1, Scale 2, Scale 3, and Scale 4. It was evident that the higher the degrees of infection, the fewer seeds per pod. The number of resistant mutants with the Scale 0, Scale 1, and Scale 2 levels of infection were three, four, and eleven, respectively. The susceptible mutants with a Scale 3 and Scale 4 grade of infection were ninety-six and nine (Figure 2A), respectively.

During the first-year field trial, the mean data on *E. polygoni*-induced single plant yield loss was assessed (Table S3) to identify the distinctive extreme mutants in the Scale 0 and 4 categories. The range of yield loss was 4.98 to 5.28% (Scale 0); 7.62 to 9.94% (Scale 1); 10.99 to 20.14% (Scale 2); 21.0 to 41.0% (Scale 3); and 42.88 to 71.86% (Scale 4). The mutants of Scale 0 and Scale 4 were utilized for the second-year confirmative trials. The climatic conditions are shown in Table S2. To assess the host physiology of *E. polygoni* infection in horse gram, the data on chlorophyll fluorescence (*a/b* ratio), number of flowering clusters per plant, number of pods per plant, number of seeds per pod, days to maturity, and single plant were recorded both under field and shade-net conditions. The results of second-

year screening experiments confirmed the findings of the first-year trial. In the Scale 0 group, the mutant TNAU-HG-M11 (RM) had minimum yield loss, and TNAU-HG-M4 (SM) exhibited the maximum yield loss in the Scale 4 category (Figure 2B,C).



**Figure 2.** (A) Categorization of horse gram mutants for powdery mildew resistance; (B) Percentage of yield loss in the resistant mutants (Scale 4) due to *E. polygoni* infection; (C) Percentage of yield loss in the susceptible mutants (Scale 4) due to *E. polygoni* infection.

No significant variation was noticed in the character expression of RM and SM between the field and shade-net experiments. The RM performed well in the field and shade-net experiments, and vice versa indicated the genetic influence (Figures S3 and S4). The net house studies and their data are utilized to discuss the result precisely, and the same was compared with field data for drawing meaningful inferences (Table 2).

**Table 2.** Effect of *E. polygoni* on the growth and yield attributing traits in horse gram (values are the mean ± SEM).

Traits	Filed Condition				Shade-Net Condition			
	Inoculated		Control		Inoculated		Control	
	SM	RM	SM	RM	SM	RM	SM	RM
1	DuG	DaG	DaG	DaG	DuG	DaG	DaG	DaG
2	0.32 ± 0.01	0.88 ± 0.01	0.86 ± 0.01	0.89 ± 0.01	0.33 ± 0.02	0.79 ± 0.02	0.77 ± 0.01	0.79 ± 0.01
3	73.80 ± 0.66	78.20 ± 1.04	74.40 ± 0.61	82.00 ± 0.63	56.20 ± 0.96	72.80 ± 0.52	74.80 ± 0.34	79.60 ± 0.46
4	44.00 ± 0.75	144.40 ± 1.00	135.20 ± 0.52	153.40 ± 0.67	61.20 ± 0.77	132.60 ± 0.46	130.40 ± 0.47	141.60 ± 0.61
5	1.40 ± 0.22	5.60 ± 0.22	5.20 ± 0.18	5.40 ± 0.22	1.20 ± 0.18	5.00 ± 0.28	4.80 ± 0.34	5.60 ± 0.22
6	74.00 ± 1.10	119.00 ± 0.63	121.00 ± 0.52	124.40 ± 0.61	72.40 ± 0.73	116.60 ± 0.46	117.80 ± 0.52	119.00 ± 0.63
7	14.12 ± 0.73	50.40 ± 0.92	52.20 ± 0.77	52.80 ± 0.77	14.60 ± 0.46	46.80 ± 0.59	50.20 ± 0.77	50.60 ± 0.61

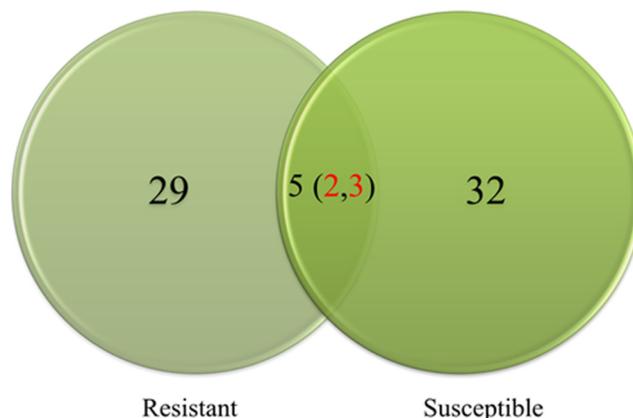
SM—Susceptible mutant. RM—Resistant mutant. DuG—Dull green. DaG—Dark green. 1—Leaf colour. 2—Chlorophyll fluorescence (a/b ratio). 3—No. flowering clusters/plant. 4—No. of pods/plant. 5—No. of seeds/pod. 6—Days to maturity. 7—Single plant yield (g).

The leaves of RM were dark-green under the inoculated condition, both in the field and shade-net experiments; on the contrary, SM had dull-green leaves. This abridged chlorophyll concentration resulted in a significant variation in chlorophyll fluorescence (a/b ratio) of SM. It was 0.33 and 0.77 under inoculated and controlled conditions, respectively, while no considerable variation was noticed in RM. The ability of *E. polygoni* to reduce the photosynthetic rate in horse gram has been reported [5]. *E. polygoni* infection resulted in a forced maturity in SM. It matured in 117.8 days in the un-inoculated condition, but it matured in 72.40 days under infested conditions. The RM maintained the same crop duration in both situations. The reduced chlorophyll fluorescence and crop duration in SM resulted in a significant variation in yield attributing traits. It had an average number of 56.20 flower-bearing clusters per plant, which led to a more substantial decrease in the associated number of pods (61.20) and number of seeds per pod (1.20). It ultimately reduced the single plant yield (14.60 g). Nevertheless, RM maintained its yield level. Therefore, these two extreme mutants were utilized to understand the powdery mildew-induced metabolic changes in horse gram.

### 3.2. Metabolomic Analysis through GC-MS Analysis

Many diseases infect plants, and thereby, significant economic loss is witnessed. Pathogen infection triggers immune response(s) in plants due to expression/activation of various metabolites. Plants with activated immune responses could survive and yield in the pathogen-challenged environments; otherwise, they succumb to the disease. In the present study, RM and SM were utilized to understand the bio-molecule production pattern in response to *E. polygoni* infection through solvent extraction followed by GC-MS. The GC-MS produced typical chromatograms between RM and SM (Figure S5a,b).

The mutants expressed both exclusive and common metabolites (Figure 3).



**Figure 3.** Number of metabolites accumulated in resistant and susceptible genotypes. Numbers in the parenthesis indicate upregulated (red) and downregulated metabolites (black).

A total of 66 bio-molecules representing 32 functional classes were expressed. The RM and SM expressed 29 and 32 exclusive metabolites, respectively. Of the five common metabolites, three were upregulated.

The details of bio-molecules and functional classes are given in Table 3 and Table S4, respectively.

The RM exhibited exclusive expression of thirteen metabolite classes while the SM expressed eleven. A total of 10 common metabolite classes, namely, alcohol, alkane, amide, amine, carboxylic acid, ester, ketone, and sugar, were expressed in SM and RM. A varied quantum of expression was noticed between the categories. The goodness of fit of the bio-molecules with the NIST library is shown in Table S5.

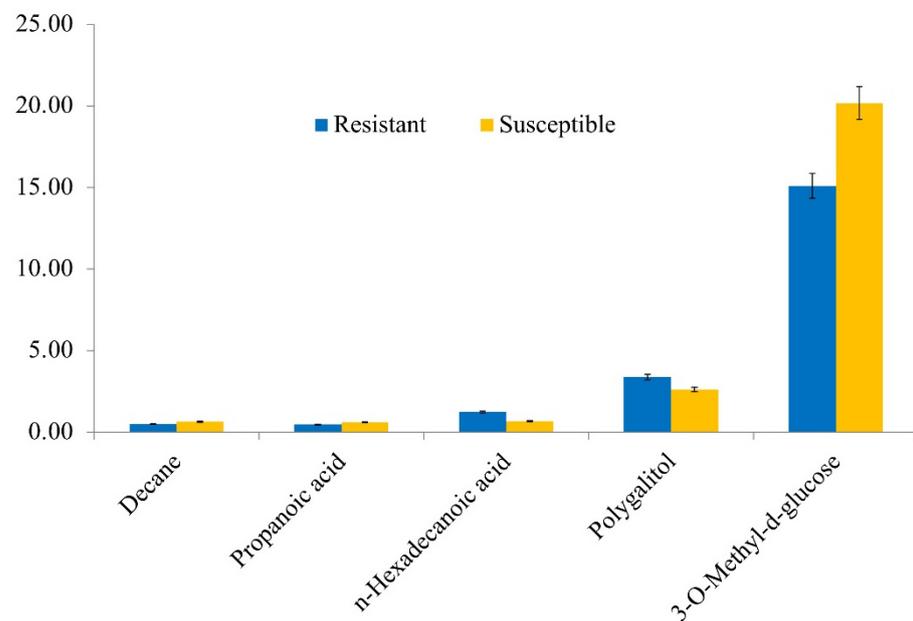
A total of seven good and two fair matches were identified in the SM, while seven good and five fair matches were observed in the RM. The bio-molecules decane, propanoic acid,

n-Hexadecanoic acid, polygalitol, and 3-o-Methyl-d-Glucose were commonly expressed in both SM and RM, and the fold change is presented in Figure 4.

**Table 3.** List of differentially expressed bio-molecules classes in horse gram with respect to powdery mildew infection.

S. No	Class	Peak Area (%)		S. No	Class	Peak Area (%)	
		R	S			R	S
1	Acetate	0.29	-	17	Furan	1.58	-
2	Alcohol	4.18	0.30	18	Glucosinolate	-	0.35
3	Aldehyde	0.27	-	19	Hydroquinone	-	1.67
4	Aliphatic nitrile	-	0.61	20	Ketone	0.79	0.86
5	Alkane	0.49	1.42	21	Nitrile	0.34	-
6	Alkene	-	0.90	22	Nitrogen compound	-	0.35
7	Amide	0.43	0.56	23	Nitrosourea	-	0.66
8	Amine	18.70	8.46	24	Organochloride	0.38	-
9	Aromatic dicarboxylic acid	0.60	-	25	Oxime	0.36	-
10	Benzodiazepines	0.27	-	26	Phenol	-	0.68
11	Benzoic acid	0.86	-	27	Piperidine	0.30	-
12	Carboxylic acid	5.30	6.5	28	Silane	0.30	-
13	Carotenoid	-	0.95	29	Siloxane	3.91	-
14	Ester	0.37	0.26	30	Silyl ether	-	2.04
15	Ether	-	0.50	31	Sugar	18.47	22.79
16	Fluorene	0.28	-	32	Terpenoid	-	0.32

S—Susceptible. R—Resistant.



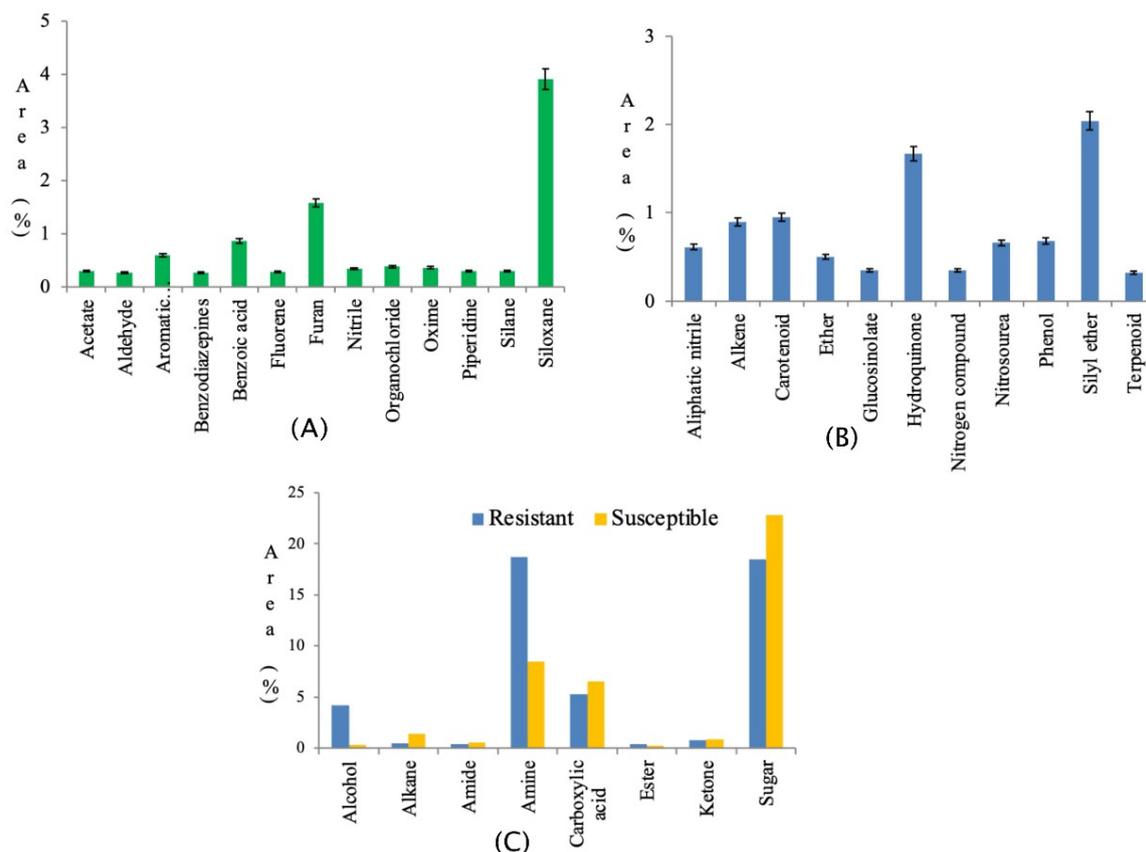
**Figure 4.** Fold change analysis of the common metabolites.

The fold change was not significant, except for 3-o-Methyl-d-Glucose (sugar) and polygalitol (alcohol). The expression of the former was significant in SM while the latter was in RM.

### 3.3. Biological Significance of Unique Metabolites Expressed in the Resistant Mutant

In RM, a total of 13 exclusive classes of compounds were expressed (Figure 5A). The order of expression was siloxane (3.91%), furan (1.58%), benzoic acid (0.86%), and aromatic dicarboxylic acid (0.60%). The other resistance-specific, narrow-ranged metabolites were

organochloride (0.38%), oxime (0.36%), nitrile (0.34%), piperidine (0.30%), acetate (0.29%), fluorene (0.28%), aldehyde (0.27%), and benzodiazepines (0.27%). These bio-molecules, either individually and/or in combination, helped the RM to resist the pathogen development, spread, and therefore finally yielded better at harvest (Table 2).



**Figure 5.** (A) Enhanced accumulation pattern of metabolites in the powdery mildew-resistant mutant; (B) Enhanced accumulation pattern of metabolites in the powdery mildew-susceptible mutant; (C) Accumulation pattern of common metabolites in the powdery mildew-resistant and -susceptible mutants.

Siloxane was the most significantly expressed bio-molecule in the RM. Siloxane or silicon (Si) are previously considered as non-essential for plant growth and development [15]; however, application of Si improved plant vigour and resistance against diseases [16]. Si imparts disease resistance through the formation of physical barriers [16], signal transduction [17], activation of defence compounds [18], and enzymes [19]. The anti-microbial properties of furan derivatives were earlier reported by [20]. The furan derivatives are synthesized in leaves constantly and exhibit strong free radical scavenging capacity [21]. Therefore, it is inferred that RM counteracted the *E. polygona*-induced oxidative stress in the leaves through a high accumulation of furan.

Plants produce a variety of benzoic acid (BA) compounds, including aromatic dicarboxylic acid, to sustain various stresses. The BAs involved in signal transduction induced systemic acquired resistance [22], regulate the stress-induced forced senescence [23], and promote and shoot growth [24] in challenging environments. Expression of organochlorine was observed in the RM. Natural halogenated compounds were isolated commonly from marine ecosystem-borne microorganisms [25]. However, very few organochlorine compounds were isolated from higher plants [26]. The synthesis of organochlorine was reported under the diseased condition in lily with antifungal activity [27].

Oximes (R<sub>1</sub>R<sub>2</sub>C=NOH) are the volatile compounds produced in response to the environment [28], and act as herbivore deterrents [29], are attractants for natural enemies [30],

and are ATP production inhibitors in microorganisms [31,32]. Oximes are converted to the corresponding alcohols, aldehydes, carboxylic acids, and glucosides, thereby conferring resistance to plants. Further, oximes regulate the production of plant hormones IAA and indole-3-acetaldoxime [33–35] and thus maintain plant growth under stress. Oxime expression was witnessed only in RM; therefore, one can hypothesize that it might have (i) helped in the regulation of auxin synthesis in RM, as it maintained good crop growth under powdery mildew infection (Table 2); and (ii) inhibited ATP production in *E. polygoni* as it failed to survive (Figures S3–S5), and part of the oximes might have been converted and stored as aldehyde. Nevertheless, aldehyde expression was not noticed in SM (Figure 4). Aldehyde-induced expression of plant defence genes was reported by [36]. On the contrary, Hansjakob [37] reported that aldehyde helped in powdery mildew spore germination.

Oximes are present in E and Z forms; the former synthesizes cyanogenic glucoside and later glucosinolate. Certain microorganisms convert Z oximes into corresponding nitriles, thereby escaping from the toxic effects of oximes [38], and therefore readily infect the plant. In the present study, *E. polygoni* converted a part of the oxime into nitrile (Table 3) in RM, but the mutant performed well with the help of other defensive metabolites. Piperidine is toxic and has antifeedant properties [39,40]. It is synthesised against herbivores in *Lobelia cardinalis* [41] and *Conium maculatum* [42]. However, in the present study, the expression of piperidine was reported against *E. polygoni*. The anti-microbial activity of acetic acid is well recognized as it influences the proton release in the membrane [43]. Expression of two unique metabolites, ‘silane’ and ‘fluorene’, were observed in RM. The biological significance of these bio-molecules in disease resistance is to be investigated. However, Lu et al. [44] reported the beneficial effect of silane in improving plant fibre quality and stability, and Roessingh et al. [45] observed the feeding and oviposition stimulant ability of fluorene.

### 3.4. Biological Significance of Unique Metabolites Expressed in the Susceptible Mutant

In SM, eleven unique metabolites were expressed (Figure 5B). The linear order of expression was silyl ether (2.04%), hydroquinone (1.67%), carotenoid (0.95%), alkene (0.90%), phenol (0.68%), nitrosourea (0.66%), aliphatic nitrile (0.61%), ether (0.5%), glucosinolate (0.35%), nitrogen compound (0.35%), and terpenoid (0.32%).

Silyl ether was the most predominant bio-molecule. Silyl ether compounds have a silicon (si) atom. Si mediates resistance to pathogen infection through signalling and defence gene expression [17], structural modifications [46], systemic acquired resistance, and production of antimicrobial compounds [18]. Si accumulates in epidermal tissue, forms complex organic compounds, and induces the production of phenolic compounds [47]. Expression of phenol was also noticed in the present quest.

Hydroquinones are a category of phenolic lipids that have salicylic acid (SA) as their phenolic group. The role of SA in disease resistance is well documented [48]. Hydroquinone inhibits disease development and improves survival and seedling vigour [49]. Terpenoids are the largest group of volatiles produced by the plants in response to insect and pathogen-induced stresses [50]. Mainly, terpenoids are produced under insect attack [51]. However, Prsic et al. [52] reported the role of terpenes as phytoalexins and their accumulation in response to pathogen infection. Carotenoids are the C40 terpenoids that play a significant role in photosynthesis, photo-protection, photomorphogenesis [53], signalling under oxidative stress [54], root development, and branching [55]. It is inferred that the SM tried to maintain the normal photosynthetic process under PM challenge through the expression of carotenoid.

The SM expressed a significant amount of alkene. The plant cuticle is made up of the long-chain fatty acid derivatives alkanes, alkenes, and secondary alcohols [56]. It provides resistance to pest and disease attacks and is associated with a few other plant developmental processes [57]. The role of alkene in disease resistance was reported by [58]. Expression of glucosinolate was observed in SM, which is a proven toxic metabolite against microorganisms and is widely reported in Brassicales [59]. A higher expression of aliphatic

nitrile than glucosinolate was also noticed, indicating the ability of *E. polygoni* to detoxify glucosinolate. Such detoxification forced SM to succumb to *E. polygoni*. Two bio-molecules, 2-phenyl-1,3-dioxan-5-yl ester (an ether molecule) and nitrosourea, were expressed only in the SM, whose role in plant defence is to be investigated. However, the anti-oxidant ability of 2-phenyl-1,3-dioxan-5-yl ester was reported in mammals [60], and nitrosourea is a well-proven alkylating agent [61], mainly used as an anti-cancer drug.

### 3.5. Biological Significance of Expression of the Common Class of Bio-Molecules Expressed Both in Resistant and Susceptible Mutants

A total of eight classes of compounds, namely, alkane, alcohol, sugar, carboxylic acid, ketone, amine, amide, and ester, were commonly expressed in RM and SM (Figure 5C).

Sugar was the most expressed metabolite, indicating its vital role in stress alleviation. Earlier, the multi-functionality of sugars in signalling, metabolic gene activation, and immunity trigger was reported by [62,63]. Further, a common expression of carboxylic acid between RM and SM made liable to affirm the earlier findings of [64] as carboxylic acid regulates the activation of the sugar signalling pathways and, thus, confers resistance to biotic stress. Amine (18.7%) and alcohol (4.18%) were highly expressed in RM. Polyamines play a vital role in plant developmental processes from root growth to seed development [65]. The functions of amines under abiotic stress [66] and biotic stress [67] are well documented. The role of various amides in plant disease resistance has also been reported previously [68].

Epidermal cuticles impart resistance to biotic and abiotic stresses. The cuticle is formed by cutin and cuticular wax. Samuels et al. [69] reported stress-induced cuticular wax modification, the subsequent production of alcohols, alkanes, esters, aldehydes, ketones, and secondary metabolites, and their protective roles. In horse gram, *E. polygoni* induced the expression of the above-listed compounds, but the quantity varied between PM and SM. Despite the expression of eight common classes of compounds, whose role in plant–microorganism interaction is well documented, the SM failed to perform well under powdery mildew infection.

## 4. Conclusions

In vivo and net house studies on powdery mildew resistance screening in horse gram yielded two extreme classes of resistant and susceptible mutants. *E. polygoni* affected the expression of yield attributing traits. The RM yielded well despite the initial infection. However, the characterized powdery mildew symptoms were noticed in leaves and pods of SM, and, therefore, it yielded very low. Comparative GC-MS analyses revealed that the RM expressed thirteen classes of unique metabolites through which it resisted pathogenicity. Although *E. polygoni* infection upregulated unique and common stress-responsive metabolites in the SM, it failed to combat the pathogenicity. Stress-triggered immune responsive functions in the SM may not be fully functional, and/or a part of the metabolites might have been diverted for other plant metabolic processes to survive in the stressed situation, which was evidenced through the presence of a few poorly filled pods and newer flowers till harvest.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12040800/s1>, Figure S1: Stages of *E. polygoni* infection in horse gram leaves; Figure S2: Stages of *E. polygoni* infection in horse gram pods (a) Maturing pods (b) Impact of infection on seed yield; Figure S3: Powdery mildew resistance as field view. (a) powdery mildew susceptible and (b) resistant horse gram mutant; Figure S4: Performance of horse gram mutants under shade net conditions. (a) powdery mildew susceptible mutant and (b) powdery mildew resistant mutant; Figure S5: Chromatograms of (a) acetone extract of powdery mildew resistant horse gram mutant and (b) acetone extract of powdery mildew susceptible horse gram mutant; Table S1: Details of horse gram mutants screened for powdery mildew resistance; Table S2: The details of climatic conditions prevailed during powdery mildew resistance screening; Table S3: The percentage of yield loss due to *E. polygoni* infection in different resistant classes of horse gram (values are in mean + SEM); Table S4: Details of bio-molecules expressed in horse gram in response

to powdery mildew infestation; Table S5: The goodness of fit of the extracted bio-molecules from powdery mildew resistant and susceptible genotypes based on SI and RSI values.

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## References

- Purushottam, R.K.; Barman, R.; Saren, B. Improvement of Neglected Horse Gram Production for Benefit of Mankind. *Int. J. Bio-Res. Environ. Agril. Sci.* **2017**, *3*, 521–527.
- Kumar, D. *Horsegram an Introduction*; Scientific Publishers: Jodhpur, India, 2006; pp. 1–10.
- National Academy of Sciences. Moth bean. In *Tropical Legumes: Resources for the Future*; The National Academies Press: Washington, DC, USA, 1978.
- Priyanka, S.; Sudhagar, R.; Vanniarajan, C.; Ganesamurthy, K.; Souframanien, J. Induction of Genetic Variability for Quantitative Traits in Horsegram (*Macrotyloma uniflorum*) through Irradiation Mutagenesis. *J. Environ. Biol.* **2021**, *42*, 597–608. [[CrossRef](#)]
- Dutta, U.; Gupta, S.; Jamwal, A.; Jamwal, S. Integrated Disease Management of Horsegram (*Macrotyloma uniflorum*). In *Diseases of Field Crops. Diagnosis and Management. Volume 2: Pulses, Oil Seeds, Narcotics, and Sugar Crops*; Srivastava, J.N., Singh, A.K., Eds.; CRC Press: Boca Raton, FL, USA, 2020; pp. 91–104.
- Ullah, N.; Yüce, M.; NeslihanÖztürkGökçe, Z.; Budak, H. Comparative Metabolite Profiling of Drought Stress in Roots and Leaves of Seven *Triticeae* Species. *BMC Genom.* **2017**, *18*, 969. [[CrossRef](#)] [[PubMed](#)]
- You, J.; Zhang, Y.; Liu, A.; Li, D.; Wang, X.; Dossa, K.; Zhou, R.; Yu, J.; Zhang, Y.; Wang, L.; et al. Transcriptomic and Metabolomic Profiling of Drought-Tolerant and Susceptible Sesame Genotypes in Response to Drought Stress. *BMC Plant Biol.* **2019**, *19*, 1–16. [[CrossRef](#)] [[PubMed](#)]
- Yang, L.; Fountain, J.C.; Ji, P.; Ni, X.; Chen, S.; Lee, R.D.; Kemerait, R.C.; Guo, B. Deciphering Drought-Induced Metabolic Responses and Regulation in Developing Maize Kernel. *Plant Biotechnol. J.* **2018**, *16*, 1616–1628. [[CrossRef](#)] [[PubMed](#)]
- Golldack, D.; Luking, I.; Yang, O. Plant Tolerance to Drought and Salinity: Stress Regulating Transcription Factors and their Functional Significance in the Cellular Transcriptional Network. *Plant Cell Rep.* **2011**, *30*, 1383–1391. [[CrossRef](#)]
- Rai, V. Role of Amino Acids in Plants Responses to Stresses. *Biol. Plant.* **2002**, *45*, 481–487. [[CrossRef](#)]
- Pal, A.B.; Brahmappa, H.S.; Rawal, R.D.; Ullasa, A. Field Resistance of Pea Germplasm to Powdery Mildew (*Erysiphe polygoni*) and Rust (*Uromyces fabae*). *Plant Dis.* **1980**, *64*, 1085–1086. [[CrossRef](#)]
- Banyal, D.K.; Tyagi, P.D. Comparison of Screening Techniques for Evaluation of Resistance among Pea Genotypes for Powdery Mildew. In *Natural Resource Management for Sustainable Hill Agriculture*, 2nd ed.; Ghabroo, S.K., Bhagat, R.M., Kapoor, A.C., Eds.; HPKV: Palampur, India, 1998; pp. 340–346.
- Banyal, D.K.; Singh, A.; Tyagi, P.D. Pathogenic Variability in *Erysiphe pisi* Causing Pea Powdery Mildew. *Himachal J. Agric. Res.* **2005**, *32*, 87–92.
- Fiehn, O.; Kopka, J.; Dormann, P.; Altmann, T.; Trethewey, R.N.; Willmitzer, L. Metabolite Profiling for Plant Functional Genomics. *Nat. Biotechnol.* **2000**, *18*, 1157–1161. [[CrossRef](#)]
- Epstein, E.; Bloom, A.J. *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd ed.; Sinauer Associates Inc.: Sunderland, UK, 2005; p. 48.
- Guerriero, G.; Hausman, J.F.; Legay, S. Silicon and the Plant Extracellular Matrix. *Front. Plant Sci.* **2016**, *7*, 463. [[CrossRef](#)] [[PubMed](#)]

17. Fauteux, F.; Remus-Borel, W.; Menzies, J.G.; Belanger, R.R. Silicon and Plant Disease Resistance against Pathogenic Fungi. *FEMS Microbiol. Lett.* **2005**, *249*, 1–6. [[CrossRef](#)] [[PubMed](#)]
18. Remus-Borel, W.; Menzies, J.G.; Bélanger, R.R. Silicon Induces Antifungal Compounds in Powdery Mildew-Infected Wheat. *Physiol. Mol. Plant Pathol.* **2005**, *66*, 108–115. [[CrossRef](#)]
19. Rahman, A.; Wallis, C.M.; Uddin, W. Silicon-Induced Systemic Defense Responses in Perennial Ryegrass against Infection by *Magnaportheorizae*. *Phytopathology* **2015**, *105*, 748–757. [[CrossRef](#)] [[PubMed](#)]
20. Hulbert, P.B.; Beuding, E.; Robinson, C.H. Structure and Antisehistosomal Activity in the Nitrofuran Series. *J. Med. Chem.* **1973**, *16*, 72. [[CrossRef](#)]
21. Spiteller, G. Furan Fatty Acids: Occurrence, Synthesis, and Reactions. Are Furan Fatty Acids Responsible for the Cardioprotective Effects of a Fish Diet? *Lipids* **2005**, *40*, 755–771. [[CrossRef](#)]
22. Malamy, J.; Carr, J.P.; Klessig, D.F.; Raskin, I. Salicylic Acid: A Likely Endogenous Signal in the Resistance Response of Tobacco to Viral Infection. *Science* **1990**, *250*, 1002–1004. [[CrossRef](#)]
23. Vlot, A.C.; Dempsey, D.A.; Klessig, D.F. Salicylic Acid, A Multifaceted Hormone to Combat Disease. *Annu. Rev. Phytopathol.* **2009**, *47*, 177–206. [[CrossRef](#)]
24. Mutui, T.M.; Mibus, H.; Serek, M. Effect of Meta-Topolin on Leaf Senescence and Rooting in *Pelargonium* × *Hortorum* Cuttings. *Postharvest Biol. Technol.* **2012**, *63*, 107–110. [[CrossRef](#)]
25. Geckeler, K.E.; Eberhardt, W. Biogenic Organochlorine Compounds—Occurrence, Function and Ecological Relevance. *Naturwissenschaften* **1995**, *82*, 2–11. [[CrossRef](#)]
26. Siuda, J.F.; DeBernardis, J.F. Naturally Occurring Halogenated Organic Compounds. *Lloydia* **1973**, *36*, 107–143.
27. Monde, K.; Satoh, H.; Nakamura, M.; Tamura, M.; Takasugi, M. Organochlorine Compounds from a Terrestrial Higher Plant: Structures and Origin of Chlorinated Orcinol Derivatives from Diseased Bulbs of *Lilium maximowiczii*. *J. Nat. Prod.* **1998**, *61*, 913–921. [[CrossRef](#)]
28. Knudsen, J.T.; Eriksson, R.; Gershenzon, J.; Stahl, B. Diversity and Distribution of Floran Scent. *Bot. Rev.* **2006**, *71*, 1–120. [[CrossRef](#)]
29. Irmisch, S.; McCormick, A.C.; Boeckler, G.A.; Schmidt, A.; Reichelt, M.; Schneider, B.; Block, K.; Schnitzler, J.P.; Gershenzon, J.; Unsicker, S.B.; et al. Two Herbivore-Induced Cytochrome P450 Enzymes CYP79D6 and CYP79D7 Catalyze the Formation of Volatile Aldoximes Involved in Poplar Defense. *Plant Cell* **2013**, *25*, 4737–4754. [[CrossRef](#)]
30. Raguso, R.A. Wake up and Smell the Roses: The Ecology and Evolution of Floral Scent. *Annu. Rev. Ecol. Evol. Syst.* **2008**, *39*, 549–569. [[CrossRef](#)]
31. Sakurada, K.; Ikegaya, H.; Ohta, H.; Fukushima, H.; Akutsu, T.; Watanabe, K. Effects of Oximes on Mitochondrial Oxidase Activity. *Toxicol. Lett.* **2009**, *189*, 110–114. [[CrossRef](#)]
32. Møller, B.L. Plant Science. Dynamic Metabolons. *Science* **2010**, *330*, 1328–1329. [[CrossRef](#)]
33. Bak, S.; Olsen, C.E.; Halkier, B.A.; Møller, B.L. Transgenic Tobacco and Arabidopsis Plants Expressing the Two Multifunctional Sorghum Cytochrome P450 Enzymes, CYP79A1 and CYP71E1, are Cyanogenic and Accumulate Metabolites Derived from Intermediates in Dhurrin Biosynthesis. *Plant Physiol.* **2000**, *123*, 1437–1448. [[CrossRef](#)]
34. Blomstedt, C.K.; Gleadow, R.M.; O'Donnell, N.; Naur, P.; Jensen, K.; Laursen, T.; Olsen, C.E.; Stuart, P.; Hamill, J.D.; Møller, B.L.; et al. A Combined Biochemical Screen and TILLING Approach Identifies Mutations in *Sorghum bicolor* L. Moench Resulting in Acyanogenic Forage Production. *Plant Biotechnol. J.* **2012**, *10*, 54–66. [[CrossRef](#)]
35. Clausen, M.; Kannangara, R.M.; Olsen, C.E.; Blomstedt, C.K.; Gleadow, R.M.; Jorgensen, K.; Bak, S.; Motawie, M.S.; Møller, B.L. The Bifurcation of the Cyanogenic Glucoside and Glucosinolate Biosynthetic Pathways. *Plant J.* **2015**, *84*, 558–573. [[CrossRef](#)]
36. Kishimoto, K.; Matsui, K.; Ozawa, R.; Takabayashi, J. Volatile C6-Aldehydes and Allo-Ocimene Activate Defense Genes and Induce Resistance against *Botrytis cinerea* in *Arabidopsis thaliana*. *Plant Cell Physiol.* **2005**, *46*, 1093–1102. [[CrossRef](#)] [[PubMed](#)]
37. Hansjakob, A.; Bischof, S.; Bringmann, G.; Riederer, M.; Hildebrandt, U. Very-Long-Chain Aldehydes Promote *Vitro in* Prepenetration Processes of *Blumeriagraminis* in a Dose and Chain Length-Dependent Manner. *New Phytol.* **2010**, *188*, 1039–1054. [[CrossRef](#)] [[PubMed](#)]
38. Pinakoulaki, E.; Koutsoupakis, C.; Sawai, H.; Pavlou, A.; Kato, Y.; Asano, Y.; Aono, S. Aldoxime Dehydratase: Probing the Heme Environment Involved in the Synthesis of the Carbon-Nitrogen Triple Bond. *J. Phys. Chem. B* **2011**, *115*, 13012–13018. [[CrossRef](#)]
39. Stermitz, F.R.; Tawara, J.N.; Boeckl, M.; Pomeroy, M.; Foderaro, T.A.; Todd, F.G. Piperidine Alkaloid Content of *Picea* (spruce) and *Pinus* (pine). *Phytochemistry* **1994**, *35*, 951–953. [[CrossRef](#)]
40. Shtykova, L.; Masuda, M.; Eriksson, C.; Sjödin, K.; Marling, E.; Schlyter, F.; Nydén, M. Latex Coatings Containing Antifeedants: Formulation, Characterization, and Application for Protection of Conifer Seedlings against Pine Weevil Feeding. *Prog. Org. Coat.* **2008**, *63*, 160–166. [[CrossRef](#)]
41. Oppel, C.B.; Dussourd, D.E.; Garimella, U. Visualizing a Plant Defense and Insect Counterploy: Alkaloid Distribution in Lobelia Leaves Trenched by a Plusiine Caterpillar. *J. Chem. Ecol.* **2009**, *35*, 625–634. [[CrossRef](#)]
42. Castells, E.; Berenbaum, M.R. Resistance of the Generalist Moth *Trichoplusiani* (Noctuidae) to a Novel Chemical Defense in the Invasive Plant *Conium maculatum*. *Chemoecology* **2007**, *18*, 11–18. [[CrossRef](#)]
43. Willamil, J.; Creus, E.; Pe'rez, J.F.; Mateu, E.; Martin-Orúe, S.M. Effect of a Microencapsulated Feed Additive of Lactic and Formic Acid on the Prevalence of Salmonella in Pigs Arriving at the Abattoir. *Arch. Anim. Nutr.* **2011**, *65*, 431–444. [[CrossRef](#)]

44. Lu, T.; Jiang, M.; Jiang, Z.; Hui, D.; Wang, Z.; Zhou, Z. Effect of Surface Modification of Bamboo Cellulose Fibers on Mechanical Properties of Cellulose/Epoxy Composites. *Compos. Part B Eng.* **2013**, *51*, 28–34. [[CrossRef](#)]
45. Roessingh, P.; Stadler, E.; Baur, R.; Hurter, J.; Ramp, T. Tarsal Chemoreceptors and Oviposition Behaviour of the Cabbage Root Fly (*Delia radicum*) Sensitive to Fractions and New Compounds of Host-Leaf Surface Extracts. *Physiol. Entomol.* **1997**, *22*, 140–148. [[CrossRef](#)]
46. Rodrigues, F.A.; Resende, R.S.; Dallagnol, L.J.; Datnoff, L.E. *Silicon Potentiates Host Defense Mechanisms against Infection by Plant Pathogens*; Springer: Cham, Switzerland, 2015; pp. 109–138.
47. Sakr, N. The Role of Silicon (Si) in Increasing Plant Resistance against Fungal Diseases. *Hell. Plant Prot. J.* **2016**, *9*, 1–15. [[CrossRef](#)]
48. Lu, H. Dissection of Salicylic Acid-Mediated Defense Signaling Networks. *Plant Signal Behav.* **2009**, *4*, 713–717. [[CrossRef](#)]
49. Elwaki, M.A.; El-Metwally, M.A. Hydroquinone, a Promising Antioxidant for Managing Seed Borne Pathogenic Fungi of Peanut. *Pak. J. Biol. Sci.* **2000**, *3*, 374–375.
50. Rasmann, S.; Hiltbold, I.; Ali, J. The Role of Root-Produced Volatile Secondary Metabolites in Mediating Soil Interactions. In *Advances in Selected Plant Physiology Aspects*; Montanaro, G., Bartolomeo, D., Eds.; InTech: Rijeka, Croatia, 2012; pp. 269–290.
51. Mumm, R.; Posthumus, M.A.; Dicke, M. Significance of Terpenoids in Induced Indirect Plant Defence against Herbivorous Arthropods. *Plant Cell Environ.* **2008**, *31*, 575–585. [[CrossRef](#)]
52. Prisc, S.; Xu, M.M.; Wilderman, P.R.; Peters, R.J. Rice contains Two Disparate Ent-Copalyl Diphosphate Synthases with Distinct Metabolic Functions. *Plant Physiol.* **2004**, *136*, 4228–4236. [[CrossRef](#)]
53. Nisar, N.; Li, L.; Lu, S.; Khin, N.C.; Pogson, B.J. Carotenoid Metabolism in Plants. *Mol. Plant* **2015**, *8*, 68–82. [[CrossRef](#)]
54. Ramel, F.; Birtic, S.; Ginies, C.; Soubigou-Taconnat, L.; Triantaphylides, C.; Havaux, M. Carotenoid Oxidation Products are Stress Signals that Mediate Gene Responses to Singlet Oxygen in Plants. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 5535–5540. [[CrossRef](#)]
55. Van Norman, J.M.; Zhang, J.; Cazzonelli, C.I.; Pogson, B.J.; Harrison, P.J.; Bugg, T.D.H.; Chan, K.X.; Thompson, A.J.; Benfey, P.N. Periodic Root Branching in *Arabidopsis* requires Synthesis of an Uncharacterized Carotenoid Derivative. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E1300–E1309. [[CrossRef](#)]
56. Von Wettstein-Knowles, P. Elongases and Epicuticular Wax Biosynthesis. *Physiol. Veg.* **1982**, *20*, 797–809.
57. Bernard, A.; Joubès, J. *Arabidopsis* Cuticular Waxes: Advances in Synthesis, Export and Regulation. *Prog. Lipid Res.* **2013**, *52*, 110–129. [[CrossRef](#)]
58. Gonzales-Vigil, E.; Hefer, C.A.; Von Loessl, M.E.; La Mantia, J.; Mansfield, S.D. Exploiting Natural Variation To uncover an Alkene Biosynthetic Enzyme in Poplar. *Plant Cell* **2017**, *29*, 2000–2015. [[CrossRef](#)] [[PubMed](#)]
59. Wittstock, U.; Kurzbach, E.; Herfurth, A.M.; Stauber, E. Glucosinolate Breakdown. In *Advances in Botanical Research*; Kopriva, S., Ed.; Academic Press: London, UK, 2016; pp. 125–169.
60. Sajid, M.; Khan, M.R.; Hammad Ismail, H.; Latif, S.; Rahim, A.A.; Mehboob, R.; Shah, S.A. Antidiabetic and Antioxidant Potential of *Alnus nitida* Leaves in Alloxan Induced Diabetic Rats. *J. Ethnopharmacol.* **2020**, *251*, 112544. [[CrossRef](#)] [[PubMed](#)]
61. Soffietti, R.; Trevisan, E.; Rudà, R. Handbook of Clinical Neurology. In *Neurologic Complications of Chemotherapy and Other Newer and Experimental Approaches*; Biller, J., Ferro, J.M., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 1199–1218.
62. Herbers, K.; Meuwly, P.; Frommer, W.B.; Metraux, J.P.; Sonnewald, U. Systemic Acquired Resistance Mediated by the Ectopic Expression of Invertase: Possible Hexose Sensing in the Secretory Pathway. *Plant Cell* **1996**, *8*, 793–803. [[CrossRef](#)] [[PubMed](#)]
63. Usadel, B.; Bläsing, O.E.; Gibon, Y.; Retzlaff, K.; Höhne, M.; Günther, M.; Stitt, M. Global Transcript Levels Respond to Small Changes of the Carbon Status during Progressive Exhaustion of Carbohydrates in *Arabidopsis* Rosettes. *Plant Physiol.* **2008**, *146*, 1834–1861. [[CrossRef](#)]
64. Baena-González, E.; Sheen, J. Convergent Energy and Stress Signalling. *Trends Plant Sci.* **2008**, *13*, 474–482. [[CrossRef](#)]
65. Watson, M.B.; Malmberg, R.L. Arginine Decarboxylase (Polyamine Synthesis) Mutants of *Arabidopsis thaliana* Exhibit Altered Root Growth. *Plant J.* **1998**, *13*, 231–239. [[CrossRef](#)]
66. Evans, P.T.; Malmberg, R.L. Do Polyamines Have Roles in Plant Development? *Annu. Rev. Plant Physiol.* **1989**, *40*, 235–269. [[CrossRef](#)]
67. Walters, D.R. Polyamines in Plant-Microbe Interactions. *Physiol. Mol. Plant Path.* **2000**, *57*, 137–146. [[CrossRef](#)]
68. López-Gresa, M.P.; Torres, C.; Campos, L.; Lisón, P.; Rodrigo, I.; Bellés, J.M.; Conejero, V. Identification of Defence Metabolites in Tomato Plants Infected by the Bacterial Pathogen *Pseudomonas syringae*. *Environ. Exp. Bot.* **2011**, *74*, 216–228. [[CrossRef](#)]
69. Samuels, L.; Kunst, L.; Jetter, R. Sealing Plant Surfaces: Cuticular Wax Formation by Epidermal Cells. *Annu. Rev. Plant Biol.* **2008**, *59*, 683–707. [[CrossRef](#)]